
High Throughput MicroRNA Profiling: Optimized Multiplex qRT-PCR at Nanoliter Scale on the Fluidigm Dynamic ArrayTM IFCs.

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Public Summary:

MicroRNAs are short RNA molecules that do not encode for proteins but rather regulate the production of proteins from messenger RNAs. Importantly, microRNAs have been implicated in a broad range of stem cell roles in both healthy and diseased tissues. MicroRNAs are also showing great promise as biomarkers of disease. Measuring microRNAs can be very complicated especially when working with small amounts of tissue, which is typically the case with human samples. Here, we describe a novel method of simultaneously measuring all microRNAs across multiple human samples with extremely robust results. The method has already been adapted by many labs studying a broad range of diseases.

Scientific Abstract:

The broad involvement of miRNAs in critical processes underlying development, tissue homeostasis and disease has led to a surging interest among the research and pharmaceutical communities. To study miRNAs, it is essential that the quantification of microRNA levels is accurate and robust. By comparing wild-type to small RNA deficient mouse embryonic stem cells (mESC), we revealed a lack of accuracy and robustness in previous published multiplex qRT-PCR techniques. Here, we describe an optimized method, including purifying away excessive primers from previous multiplex steps before singleplex real time detection, which dramatically increases the accuracy and robustness of the technique. Furthermore, we explain how performing the technique on a microfluidic chip at nanoliter volumes significantly reduces reagent costs and permits time effective high throughput miRNA expression profiling.

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